Hemoglobin Structure and Function

### Types of Hemoglobin in normal adult:
1. Hemoglobin A1 – 92% – 2 alpha and 2 beta globin chains
2. Hemoglobin A2 – 2 – 3% – 2 alpha and 2 delta globin chains
3. Hemoglobin F - 1 – 2% - 2 alpha and 2 gamma globin chains

### Biochemical composition = 52% Protein + 40% Lipid + 8% Carbohydrates

Normal length of RBC survival = 120 days in circulation

### HEMOGLOBIN OXYGEN DISSOCIATION CURVE (Hemoglobin’s affinity for oxygen)

- **Tense Form/Nonoxygenated Form (Deoxyhemoglobin)**
  - Hb Affinity
  - $\uparrow$ pH
  - $\uparrow$ HCO$_3^-$
  - $\downarrow$ Temp
  - $\uparrow$ 2,3-DPG
  - $\downarrow$ Hb Affinity

- **Relaxed Form/Oxygenated Form (Oxyhemoglobin)**
  - Hb Affinity
  - $\downarrow$ pH
  - $\downarrow$ HCO$_3^-$
  - $\uparrow$ Temp
  - $\downarrow$ 2,3-DPG
  - $\uparrow$ Hb Affinity

### Respiratory movement: allosteric changes that occur as hemoglobin releases and binds oxygen

### RBC PRESERVATION

**Goal:** provide viable and functional blood components for px.

**RBC viability = in vivo measurement of RBC survival after transfusion**

**How to know if viable:**
Collect blood from a healthy subject, label RBC with radioisotope, reinfuse and measure 24 hours after.

**FDA Criteria:**
1. 24 hour post transfusion RBC survival of more than 75%
2. Integrity throughout the shelf-life of stored RBC (Free Hb < 1% Total Hb)

**RBC STORAGE LESION**

- ↑Lactic Acid
- ↑Hb
- ↑Plasma K+
- ↑Plasma Hb
- ↓%Viable cells
- ↓Glucose
- ↓ATP
- ↓pH
- ↓2,3-DPG
- ↓O$_2$ delivered to tissue

### ANTICOAGULANT PRESERVATION SOLUTIONS

<table>
<thead>
<tr>
<th>WB &amp; RBC storage: 1 – 6 °C</th>
<th>ACD</th>
<th>CPD</th>
<th>CPD-1</th>
<th>CP2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose (g)</td>
<td>24.5</td>
<td>25.5</td>
<td>31.9</td>
<td>51.1</td>
</tr>
<tr>
<td>Adenine (g)</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Approx. vol per bag (450mL/Unit)</td>
<td>67.5</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial pH of solution without additive</td>
<td>5.0</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH of WB of initial day drawn into bag (RT)</td>
<td>7.0</td>
<td>7.2</td>
<td>7.4</td>
<td>7.3</td>
</tr>
<tr>
<td>Storage time (days at 1 – 6 C)</td>
<td>21</td>
<td>35</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

*Adenine: ATP production (extends shelf-life 21 – 35 days)*

*Dextrose: substrate for ATP production (cellular energy)*

*Citrate: Chelates Ca, prevente clotting*

*Monobasic Sodium Phosphate (maintain storage pH, adequate 2,3DPG)*

### Effects of transfusing 2,3-DPG depleted blood

- a. increased cardiac output
- b. decrease in mixed venous PO2 tension

***2,3 DPG levels in transfused blood go back to normal 6 – 24 hours post transfusion in normal healthy individuals***

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**HEMOGLOBIN STRUCTURE AND FUNCTION**

- a. 2 sets of 2 different globin chains
  - alpha, beta, gamma, delta
- b. 4 heme groups
  - heme = 1 protoporphyrin IX + ferrous iron
- c. 1,2,3-DPG

Formation of hemoglobin:
- iron is reduced and incorporated to heme
  - 1. globin dimers are formed
  - 2. heme + globin dimers
  - 3. dimmer + dimmer = tetramer
  - 4. 2,3 DPG is incorporated

**Types of Hemoglobin in normal adult:**
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3. Hemoglobin F - 1 – 2% - 2 alpha and 2 gamma globin chains

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**Integral protein | Peripheral protein**

- extend from the outer surface
- span the entire membrane to the inner cytoplasmic side of RBC

**Lipids & proteins are organized asymmetrically within RBC membrane**

Lipids are not equally distributed in the membrane bilayers.

<table>
<thead>
<tr>
<th>External Layer</th>
<th>Internal Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolipids + Choline Phospholipids</td>
<td>Amino Phospholipids</td>
</tr>
</tbody>
</table>

**Biochemical composition = 52% Protein + 40% Lipid + 8% Carbohydrates**
CONTAINERS FOR BLOOD COLLECTION
- Plastic must be sufficiently permeable to CO2 to maintain high pH storage level
  a. PVC (polyvinyl chloride) –

Issue 1: plasticizer di(ethylene)-phthalate (DEHP)-
Has been found to leach* from the plastic to the lipids of the plasma medium and RBC membranes of the blood during storage
*Alternative plasticizers that leach have been shown to stabilize the RBC membrane therefore reducing the extent of hemolysis
Issue 2: tendency to break at low temperatures
Frozen components in PVC bags should be handled with care
b. polyolefin – does not contain DEHP
c. latex- free plastic containers- for recipients with latex allergies

ADDITIVE SOLUTIONS
- Preserving solutions that are added to RBC after removal of plasma with/ without platelets.
Benefits:
  - Extend shelf-life of RBC to 42 days
  - Allows for the harvesting of more plasma and platelets from the unit
  - Produce RBC with lower viscosity that is easier to infuse

Plasma Removal = ↑Viscosity = Difficult to infuse
Additive (100mL in 450mL) = ↓Hct 70-85% to 50-60%

a) Primary bag = standard anticoagulant-preservation solution
b) Satellite bag/ Accessory bag = addition nutrient solution after plasma removal

### Table: ADDITIVE SOLUTIONS

<table>
<thead>
<tr>
<th>Days for storage</th>
<th>AS – 1 (Adsol)</th>
<th>AS – 3 (Nutricel)</th>
<th>AS – 5 (Optisol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH at 37C</td>
<td>6.6</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>ATP (%)</td>
<td>6.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2,3-DPG</td>
<td>6.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Hemolysis (%)</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Contents</td>
<td>Mannitol, Citrate, Phosphate, Mannitol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* routinely given to newborn infants and pediatric patients although some still prefer CPDA-1 RBCs due to concerns about one or more of the constituents in the additive solution

*RBC stored only with anticoagulant presrv, 2,3-DPG is depleted by the 2nd week of storage

RBC FRESHNESS
- *Used for autologous units & storage of rare blood types
- 1) Addition of a Cryoprotective agent (<6 days old)
- 2) Addition of Glycerol slowly with vigorous shaking (to permeate RBC)
- 3) Rapid freezing and stored in a freezer (-65°C)
- 4) 10 years storage
- 5) Deglycerolization (if not, thawed cells would be accompanied by hypertonic glycerol when infused, result in RBC lysis)
  - systemically replace the cryoprotectant with decreasing conc. of saline
  - w/ addition of 16% saline → 0.2% dextrose in normal saline
  - 12% saline
  - b. monitor excessive hemolysis (note the Hb conc. of the wash supernatant)
  - c. monitor unit osmolality to ensure adequate deglycerolization
*OPEN SYSTEM : broken sterility, add glycerol before freezing, or use saline solution.

1 – 6C, 24 hours

*CLOSE SYSTEM : Instrument ACP 215, Haemonetics - allows deglycerolization and thawing under closed system
- 450 mL collected with CPDA-1 frozen within 6 days can be stored after thawing at 1-6°C for 15 days

RBC REJUVENATION
- Process by which ATP & 2,3-DPG levels are restored or enhanced by metabolic alteration.

***Liquid state = can be rejuvenated 3 days after outdate.

Initial rejuvenation solution:
- phosphate, inosine, glucose, pyruvate and adenine (PIGPA)

Process:
1) Incubate RBC unit (from 450 mL unit + 50 mL rejuvenating solution) at 37°C for 1 hour
2) RBCs should be washed post-freezing deglycerolization to remove non-metabolized rejuvenation solution and extracellular potassium
3) Rejuvenated blood should be transfused within 24 hours

Advantage: Preserve selected autologous and rare units of blood for later use.

Disadvantage: cost consuming, expensive

CURRENT TRENDS
1. Development Of Improved Additive Solutions
- increase the storage period of RBCs
- trial by Hess & Greenwald used:
  - NaHCO3, NaPO4, adenine, dextrose, mannitol and NaCl with higher pH
  - 78% satisfactory- 24-hour in vivo survival after 12 weeks of storage
  - concentrated increase of ATP
  - by Högman and coworkers
  - hypotonic solution with chloride ions – increased storage for 7 weeks
  - uses CPD or half-strength CPD (increased pH)

2. Development Of Procedures To Reduce/Inactivate Pathogens In RBC Unit
- To inactivate unrecognized pathogen

Under clinical trials:
- 2 methods that utilize alkylating agents that react with nucleic acids of pathogens

Provisions for use:
- unreactive chemicals and/or breakdown products are removed after use by an absorbing material or washing of RBC
- RBC viability and function must be retained after treatment
- Toxicologic profile in animal testing should be done

3. Formation Of O-Type RBCs
- convert type A and B to O-type RBCs by using enzymes to remove the CHO moieties of A & B antigens
- clinical study showed that the B-type rbc converted to O were effective when transfused to O and A-type patients

4. RBC Substitutes / “oxygen therapeutics”
- developed for accidents, combat and surgery
- not approved due to major complications like increased BP upon infusion and toxicity

a) Hemoglobin – Based Oxygen Carriers (chemically modified Hb formulations)
  - a. traditional (human)
  - b. recombinant Hb
  - c. encapsulated Hb (bovine) : lower O2 affinity, better O2 uploading in ischemic tissue

b) Perfluorochemicals
- hydrocarbon structures in which all of the H atoms have been replaced by fluorine
- characteristics:
  - 1. chemically inert
  - 2. excellent gas solvents
- PFCs can dissolve as much as 40 – 70% of O2 per unit by volume compared to WB which dissolves 20% only
- injected as emulsions with albumin, fats or other chemicals
- Emulsions should be 0.1 – 0.2 mm for biocompatibility

### Table: ADVANTAGE vs DISADVANTAGE

<table>
<thead>
<tr>
<th>ADVANTAGE</th>
<th>DISADVANTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBOC</td>
<td></td>
</tr>
<tr>
<td>Long shelf-life</td>
<td>Shof intravascular half-life</td>
</tr>
<tr>
<td>Very stable</td>
<td>Possible toxicity</td>
</tr>
<tr>
<td>No Antigenicity (unless bovine)</td>
<td>Increased O2 affinity</td>
</tr>
<tr>
<td>No requirement for blood-typing procedure</td>
<td>Increased oncotic effect</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>PERFLUOROCARBON</td>
<td></td>
</tr>
<tr>
<td>Biological Inertness</td>
<td>Adverse clinical effects</td>
</tr>
<tr>
<td>Lack of immunogenicity</td>
<td>High O2 affinity</td>
</tr>
<tr>
<td>Easily synthesized</td>
<td>Retention in tissue</td>
</tr>
<tr>
<td>Requirement for O2 admin. When infused</td>
<td>Deep freeze storage temp</td>
</tr>
</tbody>
</table>

### Table: PLATELET PRESERVATION

NV : 150 – 350,000/μL or x10⁷/L
- involved in primary hemostasis
- circulates for 9 – 12 days, 2 – 4 um in diameter, disc-shaped
- 30% of platelets released from the BM are sequestered in the spleen
Role of Platelets in Hemostasis:
1. Initial arrest of bleeding by platelet plug formation
2. Stabilization of the hemostatic plug
3. Maintenance of vascular integrity

Clinical Use of Platelets:
1. Treatment of bleeding associated with thrombocytopenia and qualitative platelet defect
2. Prophylactic for patients prone to thrombocytopenia in cancer patients

Preparation of Platelet Concentrates (PC)
WB & Apheresis
- 20 – 24°C, up to 5 days (routine)
- 1 – 6°C, 48 hours (OK, not routine)
1. Platelet Rich Plasma
   Slow spin centrifugation followed by hard spin to concentrate platelets
2. Buffy Coat
   Buffy coat is collected then centrifuged with slow spin
Quality control:
- 5.5 x 10¹¹ platelets/unit
- pH ≥ 6.2 at expiration date
- Should be transfused within 4 hours after seal is broken; stored at 20-24°C
3. Plateletpheresis
   Platelets are harvested by drawing blood from a donor into an apheresis machine that retains the platelets and returns the rest to the donor
1 unit = 3.0 – 4.0 x 10¹¹ Platelets